

# UTILITY PATENT APPLICATION TRANSMITTAL

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David Duhl

Title

HUMAN CHROMOSOME 15 AND 16 BARDET-BIEHL  
SYNDROME POLYNUCLEOTIDES AND  
POLYPEPTIDES AND METHODS OF USE

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(Only for nonprovisional applications under 37 CFR § 1.53(b))

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO:

Box Patent Application  
Assistant Commissioner for Patents  
Washington, D.C. 202311. ☒ General Authorization Form & Fee Transmittal  
(Submit an original and a duplicate for fee processing)2. ☒ Specification [Total Pages] **38**  
(preferred arrangement set forth below)

- Descriptive Title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) (35 USC 113) [Total Sheets] **13**4. Oath or Declaration [Total Pages] **2**a. ☒ Unexecuted (original or copy)b. ☐ Copy from a prior application (37 CFR 1.63(d))  
(for continuation/divisional with Box 17 completed)

- i. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting  
inventor(s) named in the prior  
application,  
see 37 CFR 1.63(d)(2) and 1.33(b)

5. ☐ Incorporation By Reference (useable if box 4b is  
checked) The entire disclosure of the prior application,  
from which a copy of the oath or declaration is supplied  
under Box 4b, is considered to be part of the disclosure  
of the accompanying application and is hereby  
incorporated by reference therein.6. ☐ Microfiche Computer Program (Appendix)7. Nucleotide and Amino Acid Sequence Submission  
(if applicable, all necessary)

- a. ☐ Computer-Readable Copy
- b. ☐ Paper Copy (identical to computer copy)
- c. ☐ Statement verifying identity of above copies

## ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)10. ☐ English Translation Document (if applicable)11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS  
Citations12. ☐ Preliminary Amendment13. ☒ Return Receipt Postcard14. ☐ Small Entity Statement(s) ☐ Statement filed in prior application,  
Status still proper and desired15. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)16. ☒ Other: Certificate of Express Mail  
Check No. 12164 for \$ 1,092

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information below and in a preliminary amendment



Continuation



Divisional



Continuation-In-Part (CIP)

of prior Application No.: \_\_\_\_\_

Prior application information: Examiner \_\_\_\_\_

Group / Art Unit \_\_\_\_\_

Claims the benefit of Provisional Application No. 60/141,753 filed 06/30/99

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HUMAN CHROMOSOME 15 AND 16 BARDET-BIEDL SYNDROME  
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS OF USE

TECHNICAL FIELD

This invention relates to the identification and recombinant expression of  
5 human chromosome 15 and 16 Bardet-Biedl Syndrome Region (BBSR) proteins.

BACKGROUND OF THE INVENTION

Bardet-Biedl Syndrome (BBS) is a clinically and genetically heterogeneous  
autosomal recessive disorder characterized by obesity, polydactyly, hypogenitalism, retinal  
degeneration, mental retardation and heart and kidney abnormalities.

10 Elbedour et al. (1994) *Am. J. Med. Genet.* 52(2):164-169 have reported  
hypertrophy of the interventricular septum and dilated cardiomyopathy, in addition to other  
previously reported congenital heart defects associated with BBS.

BBS has been mapped to loci on several human chromosomes including  
chromosomes 3, 11, 15 and 16. These loci include the 3p12, 11q13, 15q22 and the 16q21  
15 chromosomal sites; also referred to as the BBS3, BBS1, BBS4 and BBS2 loci, respectively.  
(Bruford et al. (1997) *Genomics* 41(1):93-9; Leppert et al. (1994) *Nature Genet.* 7:108-  
112; Carmi et al. (1995) *Hum. Mol. Genet.* 4:9-13; Kwitek-Black et al. (1993) *Nature*  
*Genet.* 5:392-396; Sheffield et al. (1994) *Hum. Mol. Genet.* 3:1331-1335; Beales et al.  
(1997) *J. Med. Genet.* 34(2):92-8.).

20 Attempts to associate particular phenotypes with particular BBS loci have  
been reported. For example, Beales et al. (1997) *J. Med. Genet.* 34(2):92-8, reported that  
affected subjects linked to the BBS2 and 4 loci were significantly shorter than their parents,  
while those linked to the BBS1 locus were taller, indicating possible role for various BBS  
genes in influencing growth characteristics such as height. Carmi et al. (1995) *Hum. Mol.*  
25 *Genet.* 4:9-13, reported that BBS3 is associated with polydactyly of all four limbs while

BBS4 polydactyly is mostly confined to the hands and that BBS4 is associated with early-onset morbid obesity, while BBS2 appears as the leanest form of BBS.

Reports of specific genes involving BBS loci are sparse. Zhu et al. (1998) *Hum. Genet.* 193(6):674-680 have reported identification of a human p70s6 kinase with a possible role in BBS1 which is mapped to 11q13. Hoang et al. (1998) *Genomics*:52 (2): 219-222, have reported cloning of a C-terminal kinesin (KIFC3) that maps to human 16q13-q21 within the BBS2 region.

BBS and associated disorders have many serious effects on humans. There is a need for identifying compositions that are useful in diagnosis and treatment of such disorders.

#### SUMMARY OF THE INVENTION

The present invention discloses amino acid and nucleic acid sequences of human chromosome 15 and 16 Bardet-Biedl Syndrome Region (BBSR). The corresponding genes are referred to as Gene X, plasmolipin-like protein (PLP), ORPH-PPAR (PPAR), NT2 neuronal precursor-like (NTPL), and a seven transmembrane domain protein. The new genes and proteins are useful in the study, diagnosis and treatment of a variety of diseases including BBS and related conditions. Other indications that can be treated by the BBSR nucleotides, proteins and/or BBSR polypeptides, or agonists or antagonists include obesity, retinal degeneration, and disorders affecting the central nervous system, the heart, the kidneys, and the like.

Compositions and methods for expressing and using BBSR nucleotides and proteins are provided. The compositions comprise BBSR polypeptides and derivatives thereof, nucleotide sequences, expression cassettes, vectors, transformed cells and antibodies. Methods for the expression and detection of BBSR nucleotides and polypeptides and compositions for the treatment of BBS related conditions are provided.

The invention further provides (a) a polynucleotide encoding amino acids

from about 1 or about 2 to about 254 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids from about 1 or about 2 to about 218 of SEQ ID NO:4; (c) a polynucleotide encoding amino acids from about 1 or about 2 to about 297 of SEQ ID NO:6; (d) a polynucleotide encoding amino acids from about 1 or about 2 to about 513 of SEQ ID NO:9; (e) a polynucleotide comprising SEQ ID NO:7; (f) the polynucleotide complement of the polynucleotide of any one of (a) through (e); and (g) a polynucleotide at least 90% identical to the polynucleotide of any one of (a) through (e).

The invention still further provides a method of making a recombinant vector comprising inserting a nucleic acid molecule of (a) through (g) into a vector in operable linkage to a promoter; a recombinant vector produced thereby; a method of making a recombinant host cell comprising introducing the recombinant vector into a host cell; a recombinant host cell produced by this method; and a recombinant method of producing a polypeptide, comprising culturing the recombinant host cell under conditions such that the polypeptide is expressed, and recovering the polypeptide.

The invention also provides an isolated polypeptide comprising an amino acid sequence from (a) about 1 or about 2 to about 254 of SEQ ID NO:2; (b) about 1 or about 2 to about 218 of SEQ ID NO:4; (c) about 1 or about 2 to about 297 of SEQ ID NO:6; or (d) about 1 or about 2 to about 513 of SEQ ID NO:9; an isolated polypeptide wherein, except for at least one conservative amino acid substitution, the polypeptide has an amino acid sequence of one of (a) through (d); and an isolated polypeptide comprising amino acids at least 95% identical to an amino acid sequence of one of (a) through (d).

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides the nucleotide and amino acid sequence of the human Gene X protein (SEQ ID NO:1, 2).

Figure 2 provides the nucleotide and amino acid sequence of the human PLP cDNA (SEQ ID NO:3, 4).

Figure 3 provides the nucleotide and amino acid sequence of the human PPAR cDNA (SEQ ID NO:5, 6).

Figure 4 provides the nucleotide sequence of the human NTPL cDNA (SEQ ID NO:7).

5           Figure 5 provides the nucleotide and amino acid sequence of a human seven transmembrane receptor protein (SEQ ID NO:8, 9).

#### DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for expressing and using BBSR nucleotides, proteins and polypeptides are provided. The compositions and methods find use in the  
10   treatment of BBS and BBS-related conditions including obesity, retinal degeneration, mental retardation, central nervous system disorders, heart and kidney abnormalities, and the like. More particularly, new genes, and polypeptides encoded by the genes have been identified that are useful in the treatment of these and a variety of other conditions. The human BBSR polypeptides and cDNAs are provided in Figures 1-5 (SEQ ID NO: 1-9).

15           The compositions and methods of the invention can be used for the treatment and diagnosis of BBS, a disorder having a clinical manifestation of BBS, or any disorder that shares a clinical manifestation of BBS, so long such disorders can be diagnosed and/or treated by the methods and compositions of the invention, in a clinically or experimentally determinable manner.

20           The invention provides isolated nucleic acid molecules comprising a nucleotide sequence encoding the BBSR polypeptides whose amino acid sequences are provided in Figures 1-5, or a variant or fragment of the polypeptides. Furthermore, polynucleotides comprising antisense sequences for BBSR proteins are provided.

25           The BBSR sequences provided in Figures 1-5 (SEQ ID NO:1-9) correspond to Gene X, plasmolipin-like protein (PLP), ORPH-PPAR (PPAR), NT2 neuronal precursor-like protein (NTPL), and 7-transmembrane receptor protein, respectively.

For Gene X, the invention provides a 2850 bp cDNA (SEQ ID NO:1), which was isolated from human brain frontal cortex, and encodes an open reading frame of 762 bp (encompassing bases 214-976). Northern analysis showed that the transcript of Gene X is expressed in brain (cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen), heart, skeletal muscle, spleen, testis, pancreas. Very low expression was detected in thymus, ovary, small intestine, colon, peripheral blood leukocytes, placenta, lung, liver and kidney. The gene is located on chromosome 16.

For plasmolipin-like protein, the invention provides a 1516 bp cDNA (SEQ ID NO:3) from human brain that encodes an open reading frame of 545 bp (encompassing bases 210-755). At the nucleotide level, this sequence contains about 85% identity with human NTII-11 nerve protein and rat plasmolipin. At the amino acid level the sequence has about 89% identity with rat plasmolipin and with NTII-11. The transcript of this novel gene is expressed in brain, kidney, lung, pancreas, spleen, prostate, heart, testis, small intestine, liver, colon, skeletal muscle, placenta and ovary, but is not visible on Northern blots of thymus and peripheral blood leukocytes.

Plasmolipin is a proteolipid found on the apical surface of tubular epithelial cells of the kidney and in myelinated tracts of the brain. Addition of plasmolipin to lipid bilayers induces the formation of ion channels, which are voltage-dependent and K(+)-selective. The PLP nucleic acids and proteins of the invention find use in membrane trafficking, gap junction formation, ion transport and cell volume regulation (U.S. Patent No. 5,843,714). In addition, plasmolipin transcript levels correlate with myelination of nerve cells, as crushed nerves undergoing regeneration displayed increased transcript levels.

Proteins involved in ion transport play vital roles in excitable cells and tissues, including muscle and nerve, in which ionic changes are primarily associated with electrophysiological responses such as generation of action potentials and/or excitation-contraction coupling. It is also known that proteins involved in ion transport play important roles in tissues in which ion transport is a primary physiological function, such as kidney.

The novel plasmolipin-like gene of this invention (PLP) is implicated in Bardet-Biedl syndrome (BBS) since it falls within the region containing the chromosome 16 BBS gene. The expression pattern of the PLP gene of the invention is consistent with the pleiotropic manifestations of BBS. Furthermore, since plasmolipin is involved in ion transport and myelination, the PLP sequences of the invention may be involved in the health and proper maintenance of nerve cells/fibers, and are particularly useful for diagnosis and treatment of disorders associated with the nervous system, including central and peripheral nervous systems. Examples of such disorders include but are not limited to BBS, Laurence-Moon-Bardet-Biedl syndrome, leukodystrophy, multiple sclerosis, Charcot-Marie-Tooth neuropathy type 1a, pressure neuropathy HNPP and Dejarine-Sottas disease.

For the new transcription factor, the invention provides a 2177 bp cDNA (SEQ ID NO:5) from human retina that encodes an open reading frame of 891 bp (encompassing bases 167-1057). The transcript of the transcription factor gene of the invention appeared ~1.5 kb on Northern and was expressed in brain (cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen), heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes. The transcript contains an open reading frame of 891 bp encompassing bases 167-1057 and predicts a protein of 297 amino acids.

The sequence exhibited some homology to an apolipoprotein A1 regulatory protein. Apo A1 expression is significant in cholesterol metabolism and atherosclerosis, particularly in light of the role of apo A1 in the reverse transport of cholesterol from peripheral tissues such as coronary arteries to the liver, the primary site of cholesterol metabolism for excretion. The sequences of the invention are particularly useful for the diagnosis and treatment of disorders associated with cholesterol homeostasis, for example, but not limited to obesity, atherosclerosis, and the like. Furthermore, the expression pattern of the gene of the invention is consistent with the pleiotropic manifestations of BBS

involving obesity and cardiac disorders. It is recognized that the sequences of the invention may be used to modulate cholesterol metabolism.

For the NT2 neuronal precursor-like clone (SEQ ID NO:7), the invention provides an isolated 214 base pair fragment located on BAC 17354. Northern blots utilizing  
5 a 126 base pair probe corresponding to this fragment identified a transcript of about 2.3 kb that was present in brain (cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen), heart, placenta, lung, liver skeletal muscle, kidney, pancreas, spleen, prostate, ovary, small intestine, colon, and peripheral blood leukocytes. A slightly larger transcript, about 2.4 kb, was present in testis and no expression appeared in  
10 thymus RNA.

For human chromosome 16 seven transmembrane receptor protein, the invention provides a 3686 bp cDNA fragment (SEQ ID NO:8) from human brain (frontal cortex) that encodes an open reading frame of 1539 bp (encompassing bases 658-2193). This gene is located on human chromosome 16. Hydrophobicity analysis indicates  
15 membrane spanning regions. On Northern blots, this novel gene shows a transcript of ~4.4 kb that is expressed in brain (cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen), heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes. A less abundant transcript of ~6.2 is also present in all the  
20 tissues listed above. However, the ratio of expression of the two transcripts varied from tissue to tissue. For example, in heart the 4.4 kb transcript appeared to be expressed 10X more than the ~6.2 kb transcript, but in brain this ratio was closer to 50X. The transcript encodes a minimum of 10 exons.

This gene may relate to Bardet-Biedl syndrome since it falls within the region  
25 containing the chromosome 16 BBS gene. The 7-transmembrane domain gene can be used to develop drug treatments and therapies for obesity and retinal degeneration.

Polypeptides of the invention encompass the sequences set forth herein as well as derivatives, analogs and variants thereof. Unless otherwise indicated, variants



include substantially homologous proteins having at least about 60-65%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% or more homology to one of SEQ ID NO:2, 4, 6 or 9. It is recognized that amino acid substitutions may be made, particularly conservative substitutions. See, Bowie et al. (1990) *Science* 247:1306-1310. A variant polypeptide can differ in amino acid sequence by one or more substitutions, deletions, insertions, inversions, fusions and truncations or a combination of any of these. Variants can be naturally-occurring or can be made by recombinant means or chemical synthesis. Variant polypeptides may be fully functional or lack function in one or more activities.

10 Amino acids in the protein that are essential for function can be identified by site-directed mutagenesis, alanine-scanning mutagenesis (Cunningham et al. (1989) *Science* 244:1081-1085), etc. The resulting mutant molecules are then tested for biological activity. Critical sites for receptor binding can be determined. See, for example, Smith et al. (1992) *J. Mol. Biol.* 224:899-904; de Vos et al. (1992) *Science* 255:306-312.

15 Another aspect of the invention is a chimeric polypeptide comprising a BBSR polypeptide, or fragment thereof and a polypeptide of interest. Similarly, the invention provides a chimeric polypeptide comprising a BBSR polypeptide, or fragment thereof, fused to a polypeptide of interest. Nucleotide sequences encoding chimeric BBSR proteins and polypeptides are also provided.

20 Yet another object of the invention is to provide polynucleotides that encode the mutants, fragments, and derivatives, as well as the native BBSR proteins and polypeptides. These polynucleotides can be operably linked to heterologous promoters to form expression cassettes. The expression cassettes can be introduced into suitable host cells for expression of BBSR proteins and/or polypeptides and derivatives thereof.

25 The invention encompasses polynucleotide sequences having at least 65% sequence identity to SEQ ID NO:1, 3, 5, 7, or 8 as determined using algorithms known to those of ordinary skill in the art. A preferred but non-limiting example of a suitable algorithm is the Smith-Waterman homology search algorithm as implemented in MSPRCH

program (Oxford Molecular) using an affine gap search with the following search parameters: gap open penalty of 12, and gap extension penalty of 1. For the purpose of encoding polypeptides that vary in respect to SEQ ID NO:2, 4, 6, or 9 as described herein, the polypeptide encoded by the polynucleotides having the percent homology described  
5 above is tested for retention of biological characteristics of the native protein, and significant variation from the sequence of SEQ ID NO:1, 3, 5, or 8 may be permitted as long as the protein retains such characteristics. However, if the polynucleotide variant is used as a probe for, for example, mRNA corresponding to SEQ ID NO:1, 3, 5, 7, or 8, then percent homology should be maximized to allow specific detection of the mRNA.

10 Another object of the invention is to provide a transformed cell transiently expressing or having stably incorporated into its genome an expression vector comprising a promoter operably linked to a nucleotide sequence encoding a BBSR protein or polypeptide, or a fragment, derivative, mutant or fusion thereof.

The invention further provides methods for treating BBSR protein  
15 modulated disorders, including but not limited to Bardet-Biedl Syndrome, retinal degeneration including retinitis pigmentosa, obesity, mental retardation, renal abnormalities, diabetes and cardiovascular abnormalities. The methods comprise administering a therapeutically effective amount of a BBSR protein or polypeptide, or a derivative thereof to a subject in need of such treatment. In still another aspect, the invention provides a  
20 composition comprising BBSR protein or polypeptide or an active derivative thereof, and a pharmaceutically acceptable carrier.

The compositions of the invention comprise amino acid and nucleotide sequences for BBSR proteins. Such compositions have several uses including diagnosis and treatment of other BBSR protein-modulated disorders.

25 “BBSR protein-modulated disorders” and “BBSR protein modulated-disorders” include BBS and its various clinical manifestations including but not limited to obesity, hypogenitalism, retinal degeneration, retinis pigmentosa, polydactyly,

brachydactyly, syndactyly, mental retardation, cardiovascular and renal abnormalities, and the like.

BBSR protein-modulated disorders and BBSR protein modulated-disorders also include disorders other than BBS which include a clinical manifestation associated with BBS. For example, several human diseases exist which manifest an obesity phenotype, including but not limited to Ahlstroem syndrome, polycystic ovarian disease, Usher's, Carpenter, Prader Willi, Cohen, and Morgagni-Stewart-Monel Syndromes.

Other Examples include, in addition to BBS, other human diseases characterized by retinal degeneration including, without limitation, Bassen-Kornzweig syndrome (abetalipoproteinemia), Best disease (vitelliform dystrophy), choroidemia, gyrate atrophy, congenital amaurosis, Refsum syndrome, Stargardt disease and Usher syndrome. Other retinopathies that may benefit from administration of the compositions of the invention include age-related macular degeneration (dry and wet forms), diabetic retinopathy, peripheral vitreoretinopathies, photic retinopathies, surgery-induced retinopathies, viral retinopathies (such as HIV retinopathy related to AIDS), ischemic retinopathies, retinal detachment and traumatic retinopathy retinal

The methods and compositions of the invention can be used for the treatment and diagnosis of BBS, a disorder having a clinical manifestation of BBS, or any disorder that shares a clinical manifestation of BBS, so long such disorders can be diagnosed and/or treated by the methods and compositions of the invention, in a clinically or experimentally determinable manner.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. Factors affecting the stringency of hybridization are well known to those skilled in the art and are discussed in Sambrook et al., *Molecular Cloning: A Laboratory Manual, Second Edition*, Volume 2, Chapter 9, at page 9.50.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment,

37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions, which are nonstringent. If nonspecific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

A composition containing A is “substantially free of” B when at least about 85% by weight of the total A+B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A+B in the composition, more preferably at least about 95% or even 99% by weight.

## 25 Nucleic Acid BBSR Protein Probe Assays

mRNA levels in different cell types can be detected with nucleic acid probe assays. For example, PCR, branched DNA probe assays, or blotting techniques utilizing

nucleic acid probes substantially identical or complementary to SEQ ID NO:1, 3, 5, 7, or 8 can determine the presence of BBSR protein cDNA or mRNA.

For genomic analysis or detection of denatured DNA, the nucleic acid probes will hybridize to a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO:2, 4, 6, or 9, or the complement of a sequence encoding SEQ ID NO:1, 3, 5, 7, or 8. Though many different nucleotide sequences will encode the amino acid sequences, SEQ ID NO:2, 4, 6, or 9 is preferred because it is the actual sequence expressed in the human cells as disclosed herein. For single-stranded cDNA detection, the nucleic acid probe will hybridize to the complement of a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2, 4, 6, or 9 or to a complement of SEQ ID NO:1, 3, 5, 7, or 8. For mRNA detection, the nucleic acid probe will hybridize to SEQ ID NO:1, 3, 5, 7, or 8 or to a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2, 4, 6, or 9. The nucleic acid probe sequences need not be identical to SEQ ID NO:1, 3, 5, 7, or 8 or complements thereof.

Probes are typically at least about 15 to 20 nucleotides, more preferably at least about 30 nucleotides. The probes may be produced by synthetic procedures, such as the triester method of Matteucci et al. (1981) *J. Am. Chem. Soc.* 103:3185, or according to Urdea et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:7461, or using commercially available automated oligonucleotide synthesizers. One example of a nucleotide hybridization assay is described in Urdea et al. PCT WO92/02526 and Urdea et al. U.S. Patent No. 5,124,246, herein incorporated by reference. Other methods of hybridization and detection are known to those skilled in the art.

Alternatively, the Polymerase Chain Reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in Mullis et al. (1987) *Meth. Enzymol.* 155:335-350; U.S. Patent No. 4,683,195; and U.S. Patent No. 4,683,202, incorporated herein by reference. Also, mRNA, cDNA and genomic DNA can be detected by traditional blotting techniques described in Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (New York, Cold Spring Harbor Laboratory).

### BBSR Proteins

By "BBSR proteins" is meant the proteins and polypeptides encoded by SEQ ID NO: 1, 3, 5, 7 and 8, preferably the polypeptides having the amino acid sequence of SEQ ID NO: 2, 4, 6 or 9.

Reference to the individual BBSR proteins disclosed herein is intended to be construed to include BBSR proteins of any origin which are substantially homologous to and which are biologically equivalent to the BBSR proteins characterized and described herein. Such substantially homologous proteins may be native to any tissue or species and, similarly, biological activity can be characterized in any of a number of biological assay systems.

The term "biologically equivalent" is intended to mean that the compositions of the present invention are capable of demonstrating some or all of the same biological properties in a similar fashion, not necessarily to the same degree as the BBSR as described herein or recombinantly produced human BBSR of the invention.

By "substantially homologous" it is meant that the degree of homology of human BBSR protein to BBSR protein from any species is greater than that between a BBSR of the invention and any previously described corresponding BBSR protein.

Sequence identity or percent identity is intended to mean the percentage of same residues between two sequences, referenced to human BBSR protein when determining percent identity with non-human BBSR protein, referenced to BBSR protein when determining percent identity with non-BBSR proteins, when the two sequences are aligned using the Clustal method (Higgins et al., *Cabios* 8:189-191 (1992)) of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, WI). In this method, multiple alignments are carried out in a progressive manner, in which larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues

in the alignment, determined from a residue weight table representing the probability of a given amino acid change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment=10; gap length penalty for multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise alignment=5. The residue weight table used for the alignment program is PAM250 (Dayhoff et al., in *Atlas of Protein Sequence and Structure*, Dayhoff, Ed., NDRF, Washington, Vol. 5, suppl. 3, p. 345, 1978).

Percent conservation is calculated from the above alignment by adding the percentage of identical residues to the percentage of positions at which the two residues represent a conservative substitution (defined as having a log odds value of greater than or equal to 0.3 in the PAM250 residue weight table). Conservation is referenced to human BBSR protein when determining percent conservation with non-human BBSR protein, and referenced to BBSR when determining percent conservation with non-BBSR protein. Conservative amino acid changes satisfying this requirement are: R-K; E-D, Y-F, L-M; V-I, Q-H.

The invention provides BBSR proteins or variants thereof having one or more polymers covalently attached to one or more reactive amino acid side chains. By way of example, not limitation, such polymers include polyethylene glycol (PEG), which can be attached to one or more free cysteine sulfhydryl residues, thereby blocking the formation of disulfide bonds and aggregation when the protein is exposed to oxidizing conditions. In addition, pegylation of BBSR proteins and/or muteins is expected to provide such improved properties as increased half-life, solubility, and protease resistance. BBSR proteins and/or muteins may alternatively be modified by the covalent addition of polymers to free amino groups such as the lysine epsilon or the N-terminal amino group. It will be apparent to one skilled in the art that the methods for assaying BBSR protein biochemical and/or biological

activity may be employed in order to determine if modification of a particular amino acid residue affects the activity of the protein as desired.

It may be advantageous to improve the stability of BBSR protein by modifying one or more protease cleavage sites. Thus, the present invention provides BBSR protein variants in which one or more protease cleavage site has been altered by, for example, substitution of one or more amino acids at the cleavage site in order to create as BBSR protein variant with improved stability. Such improved protein stability may be beneficial during protein production and/or therapeutic use.

Suitable protease cleavage sites for modification are well known in the art and likely will vary depending on the particular application contemplated. For example, typical substitutions would include replacement of lysines or arginines with other amino acids such as alanine. Preferred sites to substitute would include dibasic or tribasic sites within two residues of a proline. The loss of biological activity can be tested for the appropriate BBSR protein.

BBSR protein can also include hybrid and modified forms of BBSR protein including fusion proteins and BBSR fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylations so long as the hybrid or modified form retains the biological activity of BBSR.

Fusion proteins comprising BBSR protein or a biologically active or antigenic fragment thereof can be produced using methods known in the art. Such fusion proteins can be used therapeutically or can be produced in order to simplify the isolation and purification procedures. Histidine residues can be incorporated to allow immobilized metal affinity chromatography purification. Residues EQKLISEEDL contain the antigenic determinant recognized by the myc monoclonal antibody and can be incorporated to allow myc monoclonal antibody-based affinity purification. A thrombin cleavage site can be incorporated to allow cleavage of the molecule at a chosen site; a preferred thrombin



cleavage site is residues LVPRG. Purification of the molecule can be facilitated by incorporating a sequence, such as residues SAWRHPQFGG, which binds to paramagnetic streptavidin beads. Such embodiments are described in WO 97/25345, which is incorporated by reference.

5           The invention also includes fragments of BBSR proteins. Such fragments can be prepared from the protein by standard biochemical methods or by expressing a polynucleotide encoding the fragment. Also included with the scope of the invention are BBSR protein molecules that differ from native BBSR proteins by virtue of changes in biologically active sites.

10           Also included within the meaning of substantially homologous is any BBSR protein which may be isolated by virtue of cross-reactivity with antibodies to the BBSR described herein or whose encoding nucleotide sequences including genomic DNA, mRNA or cDNA may be isolated through hybridization with the complementary sequence of genomic or subgenomic nucleotide sequences or cDNA of the BBSR protein herein or  
15 fragments thereof. It will also be appreciated by one skilled in the art that degenerate DNA sequences can encode human BBSR proteins and these are also intended to be included within the present invention as are allelic variants of BBSR proteins.

          The DNA encoding BBSR proteins can be engineered to take advantage of preferred codon usage of host cells. Codon usage in *Pseudomonas aeruginosa* is described  
20 in, for example, West et al., *Nucleic Acids Res.* 11:9323-9335 (1988). Codon usage in *Saccharomyces cerevisiae* is described in, for example, Lloyd et al., *Nucleic Acids Res.* 20:5289-5295 (1992). Codon preference in *Corynebacteria* and a comparison with *E. coli* preference is provided in Malubres et al., *Gene* 134:15-24 (1993). Codon usage in *Drosophila melanogaster* is described in, for example, Akashi, *Genetics* 136:927-935  
25 (1994).

          Any suitable expression vector may be employed to produce recombinant human BBSR proteins such as expression vectors for use in insect cells. Baculovirus expression systems can also be employed.

The present invention includes nucleic acid sequences including sequences that encode human BBSR proteins. Also included within the scope of this invention are sequences that are substantially the same as the nucleic acid sequences encoding BBSR proteins. Such substantially the same sequences may, for example, be substituted with  
5 codons more readily expressed in a given host cell such as *E. coli* according to well known and standard procedures. Such modified nucleic acid sequences are included within the scope of this invention.

Specific nucleic acid sequences can be modified by those skilled in the art and, thus, all nucleic acid sequences that code for the amino acid sequences of BBSR  
10 proteins can likewise be so modified. The present invention thus also includes nucleic acid sequence which will hybridize with all such nucleic acid sequences — or complements of the nucleic acid sequences where appropriate — and encode a polypeptide having the neuronal cell survival promoting activities disclosed herein. The present invention also includes nucleic acid sequences that encode polypeptides that have neuronal cell survival promoting  
15 activity and that are recognized by antibodies that bind to BBSR proteins.

The present invention also encompasses vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the invention. This invention also includes host cells of any variety that have been transformed with vectors comprising expression regulatory elements operably linked to any  
20 of the nucleic acid sequences included within the scope of the present invention.

#### Expression of BBSR Protein and BBSR Polypeptides

Preferably, BBSR proteins and polypeptides are produced by recombinantly engineered host cells. These host cells are constructed by the introduction of a expression vector comprising a promoter operably linked to a BBSR protein or polypeptide coding  
25 sequence.

Such coding sequences can be constructed by synthesizing the entire gene or by altering existing BBSR protein or polypeptide coding sequences. BBSR polypeptides

can be divided into four general categories: mutants, fragments, fusions, and the native BBSR polypeptides. The native BBSR polypeptides are those that occur in nature. The amino acid sequence of such polypeptides may vary slightly from SEQ ID NO:2, 4, 6, and 9. The native BBSR protein and BBSR polypeptide coding sequence can be selected based on the amino acid sequence shown in SEQ ID NO:2, 4, 6, and 9. For example, synthetic genes can be made using codons preferred by the host cell to encode the desired polypeptide. (See Urdea et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:7461). Alternatively, the desired native BBSR polypeptide coding sequences can be cloned from nucleic acid libraries. Techniques for producing and probing nucleic acid sequence libraries are described, for example, in Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (New York, Cold Spring Harbor Laboratory). Other recombinant techniques, such as site specific mutagenesis, PCR, enzymatic digestion and ligation, can also be used to construct the desired BBSR protein or polypeptide coding sequence.

The native BBSR polypeptide coding sequences can be modified to create the other classes of BBSR polypeptides. For example, mutants can be created by making conservative amino acid substitutions that maintain or enhance native BBSR protein or the protein's activity. The following are examples of conservative substitutions: Gly  $\leftrightarrow$  Ala; Val  $\leftrightarrow$  Ile  $\leftrightarrow$  Leu; Asp  $\leftrightarrow$  Glu; Lys  $\leftrightarrow$  Arg; Asn  $\leftrightarrow$  Gln; and Phe  $\leftrightarrow$  Trp  $\leftrightarrow$  Tyr. Mutants can also contain amino acid deletions or insertions compared to the native BBSR polypeptides. Mutants may include substitutions, insertions, and deletions of the native polypeptides.

Mutants will retain at least about 20% of the one of the activities of the native BBSR protein. The coding sequence of mutants can be constructed by *in vitro* mutagenesis of the native coding sequences.

Fragments differ from mutant or native BBSR polypeptides by amino and/or carboxyl terminal amino acid deletions. The number of amino acids that are truncated is not critical as long as the BBSR protein fragment retains at least about 20% of the one of the activities of the native BBSR polypeptide. The coding sequence of such fragments can be

easily constructed by cleaving the unwanted nucleotides from the mutant or native BBSR polypeptide coding sequences.

Fusions are fragments, mutants, or native BBSR polypeptides with additional amino acids at either or both of the termini. The additional amino acid sequence is not necessarily homologous to sequence found in native polypeptides. The fusions, just as all BBSR polypeptides, retain at least about 20% of one of the activities of the native BBSR polypeptides. Coding sequence of the fusions can be constructed by ligating synthetic polynucleotides encoding the additional amino acids to fragment, mutant, or native coding sequences. Activities of the BBSR polypeptides can be determined by the methods described *infra*.

At the minimum, an expression vector will contain a promoter which is operable, that is drives expression in the host cell and operably linked to a BBSR protein or polypeptide coding sequence. Sequences that modulate gene expression, such as enhancers and binding sites for inducers or repressors may be present. Expression vectors may also include signal sequences, terminators, selectable markers, origins of replication, and sequences homologous to host cell sequences. These additional elements are optional but can be included to optimize expression. Construction of expression vectors is known in the art and any appropriate methods can be employed with the polynucleotides of the invention.

A BBSR protein or polypeptide coding sequence may also be linked in reading frame to a signal sequence. The signal sequence fragment typically encodes a peptide comprised of hydrophobic amino acids which directs the BBSR protein or polypeptide to the cell membrane or other subcellular compartment. Preferably, there are processing sites encoded between the leader fragment and the gene or fragment thereof that can be cleaved either *in vivo* or *in vitro*. DNA encoding suitable signal sequences can be derived from genes for secreted endogenous host cell proteins, such as the yeast invertase gene (EP 12 873; JP 62,096,086), the A-factor gene (U.S. Patent No. 4,588,684), interferon signal sequence (EP 60 057).

- After vector construction, the desired BBSR protein and/or BBSR polypeptide expression vector is inserted into the host cell. Many transformation techniques exist for inserting expression vectors into bacterial, yeast, insect, and mammalian cells. The transformation procedure to introduce the expression vector depends upon the host to be
- 5 transformed. Such methods are known in the art. See, e.g., (Masson et al. (1989) *FEMS Microbiol. Lett.* 60:273; Palva et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP Publ. Nos. 036 259 and 063 953; PCT WO 84/04541, *Bacillus*), (Miller et al. (1988) *Proc. Natl. Acad. Sci.* 85:856; Wang et al. (1990) *J. Bacteriol.* 172:949, *Campylobacter*), (Cohen et al. (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower et al. (1988) *Nuc. Acids Res.* 16:6127;
  - 10 Kushner et al. (1978) "An Improved Method for Transformation of *Escherichia coli* with ColE1-derived plasmids in *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel et al. (1970) *J. Mol. Biol.* 53:159; Taketo et al. (1988) *Biochim. Biophys. Acta* 949:318; *Escherichia*, (Chassy et al. (1987) *FEMS Microbiol. Lett.* 44:173, *Lactobacillus*); (Fiedler et al. (1988)
  - 15 *Anal. Biochem.* 170:38, *Pseudomonas*); (Augustin et al. (1990) *FEMS Microbiol. Lett.* 66:203, *Staphylococcus*), Barany et al. (1980) *J. Bacteriol.* 144:698; Harlander et al. (1987) "Transformation of *Streptococcus lactis* by electroporation," in *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss, III); Perry et al. (1981) *Infec. Immun.* 32:1295; Powell et al. (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti et al. (1987) *Proc. 4th Evr. Cong.*
  - 20 *Biotechnology* 1:412, *Streptococcus*).

Transformation methods for yeast hosts are well-known in the art, and typically include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Electroporation is another means for transforming yeast hosts. See, for example, *Methods in Enzymology*, Volume 194, 1991, "Guide to Yeast Genetics and

- 25 Molecular Biology." Transformation procedures usually vary with the yeast species to be transformed. See, e.g., (Kurtz et al. (1986) *Mol. Cell. Biol.* 6:142; Kunze et al. (1985) *J. Basic Microbiol.* 25:141, *Candida*); (Gleeson et al. (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp et al. (1986) *Mol. Gen. Genet.* 202:302, *Hansenula*); (Das et al. (1984) *J.*

*Bacteriol.* 158:1165; De Louvencourt et al. (1983) *J. Bacteriol.* 154:1165; Van den Berg et al. (1990) *Biotechnology* 8:135, *Kluyveromyces*); (Cregg et al. (1985) *Mol. Cell. Biol.* 5:3376; Kunze et al. (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,955, *Pichia*); (Hinnen et al. (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito et al. 5 (1983) *J. Bacteriol.* 153:163, *Saccharomyces*); (Beach and Nurse (1981) *Nature* 300:706, *Schizosaccharomyces*); (Davidow et al. (1985) *Curr. Genet.* 10:39; Gaillardin et al. (1985) *Curr. Genet.* 10:49, *Yarrowia*).

Methods for introducing heterologous polynucleotides into mammalian cells are known in the art and include viral infection, dextran-mediated transfection, calcium 10 phosphate precipitation, microparticle bombardment, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

#### Monitoring BBSR Polypeptide Expression Levels

Immunoassays and ligand binding assays can be utilized to confirm that the 15 transformed host cell is expressing the desired BBSR polypeptide. Polyclonal or monoclonal antibodies to BBSR proteins can be prepared by any methods known in the art, using as immunogen the whole BBSR protein or an epitope-bearing portion thereof, which can comprise between about 10 and 100 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:9, preferably between about 12 and 50 contiguous 20 amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:9, more preferably between about 15 and 25 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:9.

For example, an immunofluorescence assay can be performed on transformed host cells without separating the BBSR polypeptides from the cell. The host cells are first 25 fixed onto a solid support, such as a microscope slide or microtiter well. This fixing step permeabilizes the cell membrane. Next, the fixed host cells are exposed to an anti-BBSR polypeptide antibody. Preferably, to increase the sensitivity of the assay, the fixed cells are

exposed to a second antibody, which is labeled and binds to the anti-BBSR polypeptide antibody. Typically, the secondary antibody is labeled with a fluorescent marker. The host cells, which express the BBSR polypeptides, will be fluorescently labeled and easily visualized under the microscope. See, for example, Hashido et al. (1992) *Biochem & Biophys. Res. Comm.* 187(3):1241-1248.

Also, the BBSR polypeptides do not need to be separated from the cell membrane for *in vitro* assays. The host cells may be fixed to a solid support, such as a microtiter plate. Alternatively, a crude membrane fraction can be separated from lysed host cells by centrifugation (See Adachi et al. (1992) *FEBS Lett* 311(2):179-183. The fixed host cells or the crude membrane fraction is exposed to labeled ligand or ion. Typically, the ligand is labeled with radioactive atoms. The host cells, which express the desired BBSR polypeptide, will bind with the labeled ligand, which can be easily detected.

BBSR polypeptides can be purified and are useful as compositions, for assays, and to produce antibodies. BBSR polypeptides can be isolated by a variety of steps including, for example, anion exchange chromatography, size exclusion chromatography, hydroxylapatite chromatography, hydrophobic interaction chromatography, metal chelation chromatography, reverse phase HPLC, affinity chromatography, and further ammonium sulfate precipitations. These techniques are well known to those of skill in the art.

For ligand binding studies, patch clamp analysis or other *in vitro* assays, the crude cell membrane fractions can be utilized. These membrane extracts can be isolated from cells, which expressed BBSR polypeptides by lysing the cells. Alternatively, whole cells, expressing BBSR polypeptides, can be cultured in a microtiter plate.

### Antibodies

Antibodies against BBSR polypeptides are useful for affinity chromatography, immunofluorescent assays, and distinguishing BBSR polypeptides; and for inhibiting or modulating an activity or biological effect or a disorder associated with the BBSR-proteins of the invention. Such uses include but are not limited to modulation of

BBSR-mediated disorders; modulation of transcription, particularly that of apo A1, modulation of ion transport, cholesterol homeostasis, and the like.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods known to those skilled in the art. For example,  
5 monoclonal antibodies are prepared using the method of Kohler et al. (1975) *Nature* 256:495-496, or a modification thereof.

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly  $^{32}\text{P}$  and  $^{125}\text{I}$ ), electron-dense reagents, enzymes, and ligands  
10 having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific  
15 therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example,  $^{125}\text{I}$  may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme  
20 or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with  $^{125}\text{I}$ , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the  
25 scope of the instant invention.



### Ion Transport Activity

The channel forming and ion transport activity of the plasmolipin-like polypeptide (SEQ ID NO:4) is determined essentially as described in U.S. Patent No. 5,843,714. The plasmolipin-like polypeptide is assayed by monitoring its effect on transmembrane pH gradients in liposomes. Mitochondrial cytochrome C oxidase, a proton pump, is reconstituted into liposomes by sonication. The pH-sensitive fluorescent dye pyranine (Eastman Kodak) is then incorporated into the proteoliposomes by rapid freeze-thawing and sonication. Excess dye is removed by centrifugation and resuspension of the liposomes into an appropriate buffer. Addition of ascorbate and cytochrome C initiates proton uptake into the liposomes. PLP protein is added and proton efflux is monitored by the fluorescence changes arising from changes in internal pH of the liposomes at excitation and emission wavelengths of 460 nm and 508 nm, respectively.

Lipid bilayer destabilization promoted by the plasmolipin-like polypeptide, incorporated into membranes by expression or by reconstitution, is assayed by measurement of the fluorescence polarization of the lipophilic dye 1,6-diphenyl-1,3,5-hexatriene (Eastman Kodak) inserted into the membranes.

### Screening for Agonists and Antagonists

BBSR polypeptides can also be used to screen combinatorial libraries to identify agonist or antagonists. For example, a "library" of peptides may be synthesized following the methods disclosed in U.S. Patent No. 5,010,175, and in PCT WO 91/17823, both incorporated herein by reference in full. The peptide library is first screened for binding to the selected BBSR polypeptide. The peptides are then tested for their ability to inhibit or enhance the particular BBSR protein activity. Peptides exhibiting the desired activity are then isolated and sequenced.

Agonists or antagonists of BBSR proteins may be screened using any available method. The assay conditions ideally should resemble the conditions under which the activity of the particular BBSR protein is exhibited *in vivo*, *i.e.*, under physiologic pH,

temperature, ionic strength, etc. Suitable agonists or antagonists will exhibit strong inhibition or enhancement of the particular activity of the BBSR protein at concentrations, which do not raise toxic side effects in the subject. Agonists or antagonists which compete for binding to the BBSR polypeptide may require concentrations equal to or greater than the native BBSR protein concentration, while inhibitors capable of binding irreversibly to the polypeptide may be added in concentrations on the order of the native BBSR protein concentration.

#### Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or polynucleotides of the claimed invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the polypeptide or DNA construct in the individual to which it is administered

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for

administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly  
5 metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the  
10 salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences* (1991) (Mack Pub. Co., NJ ).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such  
15 as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

## 20 Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be mammals or birds. In particular, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by  
25 injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary

administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Alternatively, the BBSR polypeptides could be stably expressed in an organ of a mammal, and then the organ could be xenografted into a human in need of such  
5 treatment.

### Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize  
10 viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector  
15 and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. *See generally*, Jolly et al. (1994) *Cancer Gene Therapy* 1:51-64; Kimura et al. (1994) *Human Gene Therapy* 5:845-852; Connelly et al. (1995) *Human Gene Therapy* 6:185-193;  
20 and Kaplitt et al. (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and it is contemplated that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (*see* O'Neill (1985) *J. Vir.* 53:160) polytropic retroviruses (for example, MCF and MCF-MLV  
25 (*see* Kelly et al. (1983) *J. Vir.* 45:291), spumaviruses and lentiviruses. *See RNA Tumor Viruses*, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

5           These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (*see* US Serial No. 07/800,921, filed November 29, 1991). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle. *See*, US Serial No. 08/445,466 filed May 22,  
10 1995. It is preferable but not required that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (*see* US Serial No. 08/240,030, filed May 9, 1994; *see also* WO 92/05266), and can be used to create producer cell lines (also  
15 termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (*e.g.*, HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell  
20 Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley et al. (1976) *J. Virol.* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No. VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-  
25 190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO 93/09239.

Also contemplated are alpha virus gene therapy vectors that can be employed  
5 in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in U.S. patents 5,091,309, 5,217,879, and WO 92/10578, WO 95/07994, U.S. 5,091,309 and  
10 U.S. 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (*see* co-owned U.S. Serial No. 08/679640).

15

### EXAMPLES

The example presented below is provided as a further guide to the practitioner of ordinary skill in the art, and is not to be construed as limiting the invention in any way.

#### EXAMPLE 1

20

#### Polynucleotides that map to regions of the human genome associated with BBS.

A 2850 bp cDNA (SEQ ID NO:1) from human brain (frontal cortex) that encodes an open reading frame of 762 bp (encompassing bases 214-976) was isolated and is referred to herein as Gene X. The Gene X gene is located on human chromosome 16.  
25 Northern analysis showed that the transcript of Gene X is expressed in brain (cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen), heart, skeletal muscle, spleen, testis, and pancreas, with very low expression in

thymus, ovary, small intestine, colon, peripheral blood leukocytes, placenta, lung liver and kidney. The transcript corresponds to a minimum of 4 exons.

A 1516 bp cDNA (SEQ ID NO:3) was isolated from human brain that encodes an open reading frame of 545 bp (encompassing bases 210-755 bp nucleotide).

- 5 This gene is located on human chromosome 16. The nucleotide sequence shares homology with rat plasmolipin. At the amino acid level it has about 89% identity with rat plasmolipin and with NTII-11. The transcript of this novel gene is expressed in brain, kidney, lung, pancreas, spleen, prostate, heart, testis, small intestine, liver, colon, skeletal muscle, placenta and ovary, but is not visible on Northern of thymus and peripheral blood leukocytes.

- 10 A 2177 bp cDNA (SEQ ID NO:5) was isolated from human retina that encodes an open reading frame of 891 bp (encompassing bases 167-1057). This gene is located on human chromosome 15. At the nucleotide level it contains over 70% identity with several transcription factors (*e.g.*, tailless, chick ovalbumin upstream promoter transcription factor II, apolipoprotein AI regulatory protein) over regions spanning 224 bp.

- 15 The transcript of this novel gene is about 1.5 kb on Northern and was expressed in brain (cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen), heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes. The transcript contains an open reading frame of 891 bp encompassing bases 167-1057 and predicts a  
20 protein of 297 amino acids.

- Two fragments of 214 bp (SEQ ID NO:7) and 65 bp were identified which are located on BAC 17354 (from human chromosome 16). A 126 bp fragment was isolated for use as a probe for hybridization on Northern blots and this probe identified a transcript of ~2.3 kb that was present in brain (cerebellum, cerebral cortex, medulla, spinal cord,  
25 occipital lobe, frontal lobe, temporal lobe and putamen), heart, placenta, lung, liver skeletal muscle, kidney, pancreas, spleen, prostate, ovary, small intestine, colon, and peripheral blood leukocytes. A slightly larger transcript, ~2.4 kb, was present in testis and no expression appeared in thymus RNA.

Identities were determined using MPSRCH™ software, Release 3.3A (distributed by Oxford Molecular Ltd.). Nucleotide identity determinations were made using MPSRCH\_nn, using Smith-Waterman algorithm using default TABLE Gap 6.

Protein identity determinations were made either using MPsrch-PP, using  
5 Smith-Waterman algorithm, using blosum 60 TABLE, Gap 14; or by using blosum 60  
TABLE, Base Gap open 24, Gap extend 3, A-A Gap open 30, Gap extend 9.

All publications and patent applications mentioned in the specification are  
indicative of the level of those skilled in the art to which this invention pertains. All  
publications and patent applications are herein incorporated by reference to the same extent  
10 as if each individual publication or patent application was specifically and individually  
indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way  
of illustration and example for purposes of clarity of understanding, it will be obvious that  
certain changes and modifications may be practiced within the scope of the appended claims.



## CLAIMS

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

- (a) a polynucleotide encoding amino acids from about 1 to about 254 of SEQ ID NO:2;
- (b) a polynucleotide encoding amino acids from about 2 to about 254 of SEQ ID NO:2;
- (c) a polynucleotide encoding amino acids from about 1 to about 218 of SEQ ID NO:4;
- (d) a polynucleotide encoding amino acids from about 2 to about 218 of SEQ ID NO:4;
- (e) a polynucleotide encoding amino acids from about 1 to about 297 of SEQ ID NO:6;
- (f) a polynucleotide encoding amino acids from about 2 to about 297 of SEQ ID NO:6;
- (g) a polynucleotide encoding amino acids from about 1 to about 513 of SEQ ID NO:9;
- (h) a polynucleotide encoding amino acids from about 2 to about 513 of SEQ ID NO:9;
- (i) a polynucleotide consisting of SEQ ID NO:7;
- (j) the polynucleotide complement of the polynucleotide of any one of (a) through (i); and
- (k) a polynucleotide at least 90% identical to the polynucleotide of any one of (a) through (j).

2. An isolated nucleic acid molecule comprising about 762 contiguous nucleotides from the coding region of SEQ ID NO:1, about 545 contiguous nucleotides from the

coding region of SEQ ID NO:3, about 891 contiguous nucleotides from the coding region of SEQ ID NO:5, or about 1539 contiguous nucleotides from the coding region of SEQ ID NO:8.

3. An isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

- (a) amino acids from about 1 to about 254 of SEQ ID NO:2;
- (b) amino acids from about 2 to about 254 of SEQ ID NO:2;
- (c) amino acids from about 1 to about 218 of SEQ ID NO:4;
- (d) amino acids from about 2 to about 218 of SEQ ID NO:4;
- (e) amino acids from about 1 to about 297 of SEQ ID NO:6;
- (f) amino acids from about 2 to about 297 of SEQ ID NO:6;
- (g) amino acids from about 1 to about 513 of SEQ ID NO:9;
- (h) amino acids from about 2 to about 513 of SEQ ID NO:9.

4. The isolated nucleic acid molecule of claim 1, which is DNA.

5. A method of making a recombinant vector comprising inserting a nucleic acid molecule of claim 1 into a vector in operable linkage to a promoter.

6. A recombinant vector produced by the method of claim 5.

7. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 6 into a host cell.

8. A recombinant host cell produced by the method of claim 7.

9. A recombinant method of producing a polypeptide, comprising culturing the recombinant host cell of claim 8 under conditions such that said polypeptide is expressed and recovering said polypeptide.

10. An isolated polypeptide comprising amino acids at least 95% identical to amino acids selected from the group consisting of:

- (a) amino acids from about 1 to about 254 of SEQ ID NO:2;
- (b) amino acids from about 2 to about 254 of SEQ ID NO:2;
- (c) amino acids from about 1 to about 218 of SEQ ID NO:4;
- (d) amino acids from about 2 to about 218 of SEQ ID NO:4;
- (e) amino acids from about 1 to about 297 of SEQ ID NO:6;
- (f) amino acids from about 2 to about 297 of SEQ ID NO:6;
- (g) amino acids from about 1 to about 513 of SEQ ID NO:9;
- (h) amino acids from about 2 to about 513 of SEQ ID NO:9.

11. An isolated polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

- (a) amino acids from about 1 to about 254 of SEQ ID NO:2;
- (b) amino acids from about 2 to about 254 of SEQ ID NO:2;
- (c) amino acids from about 1 to about 218 of SEQ ID NO:4;
- (d) amino acids from about 2 to about 218 of SEQ ID NO:4;
- (e) amino acids from about 1 to about 297 of SEQ ID NO:6;
- (f) amino acids from about 2 to about 297 of SEQ ID NO:6;
- (g) amino acids from about 1 to about 513 of SEQ ID NO:9;
- (h) amino acids from about 2 to about 513 of SEQ ID NO:9.

12. An isolated polypeptide comprising amino selected from the group consisting of:

- (a) amino acids from about 1 to about 254 of SEQ ID NO:2;
- (b) amino acids from about 2 to about 254 of SEQ ID NO:2;
- (c) amino acids from about 1 to about 218 of SEQ ID NO:4;
- (d) amino acids from about 2 to about 218 of SEQ ID NO:4;
- (e) amino acids from about 1 to about 297 of SEQ ID NO:6;
- (f) amino acids from about 2 to about 297 of SEQ ID NO:6;
- (g) amino acids from about 1 to about 513 of SEQ ID NO:9;
- (h) amino acids from about 2 to about 513 of SEQ ID NO:9.

13. An epitope-bearing portion of a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:9.

14. The epitope-bearing portion of claim 13, which comprises between about 10 and 100 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:9.

15. The epitope-bearing portion of claim 14, which comprises between about 12 and 50 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:9.

16. The epitope-bearing portion of claim 14, which comprises between about 15 and 25 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:9.

17. An isolated antibody that binds specifically to the polypeptide of claim 10.

18. An isolated antibody that binds specifically to the polypeptide of claim 11.
19. An isolated antibody that binds specifically to the polypeptide of claim 12.
20. A method for diagnosing a BBSR protein-modulated disorder using a biological sample from a human suspected of having said disorder, said method comprising :
  - a) providing an antibody that binds to the polypeptide of claim 10;
  - b) contacting the antibody with said sample under binding conditions to form a duplex; and
  - c) determining the amount of said duplex formed, compared to a normal sample.
21. A method for diagnosing a BBSR protein-modulated disorder in a biological sample from a human suspected of having said disorder, said method comprising:
  - a) providing a polynucleotide that binds to mRNA encoding the polypeptide of claim 10 under stringent conditions;
  - b) contacting nucleic acid of said sample with said polynucleotide under binding conditions to form a duplex; and
  - c) determining the amount of said duplex formed, compared to a normal sample.
22. A method for modulating the amount of a BBSR protein in a subject, said method comprising administering an effective amount of a composition selected from a group consisting of:
  - a) the polypeptide according to claim 10; and
  - b) an antibody that binds to the polypeptide according to claim 10.

23. A method for modulating the amount of a BBSR protein in a subject, said method comprising administering an effective amount of a composition consisting of the nucleotide sequence according to claim 1.

24. A method for treating a BBSR protein-modulated disorder in a subject, said method comprising administering to said subject an effective amount of a composition selected from a group consisting of:

- a) the polypeptide according to claim 10; and
- b) an antibody that binds to the polypeptide according to claim 10; wherein said composition further comprises a pharmaceutically acceptable carrier.

25. A method for treating a BBSR protein-modulated disorder in a subject, said method comprising administering to said subject an effective amount of a composition consisting of the nucleotide sequence according to claim 1, wherein said composition further comprises a pharmaceutically acceptable carrier.

HUMAN CHROMOSOME 15 AND 16 BARDET-BIEDL SYNDROME  
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS OF USE

ABSTRACT OF THE DISCLOSURE

Amino acid and nucleic acid sequences of Bardet-Biedl Syndrome Region (BBSR) that map to human chromosome 15 or 16 are disclosed. Compositions and methods related to the genes and proteins are useful in the study, diagnosis and treatment of a variety of diseases including BBS and related conditions including obesity, retinal degeneration, and disorders affecting the nervous system, the heart, the kidneys, and the like. Compositions include those comprising BBSR polypeptides and derivatives thereof, nucleotide sequences, expression cassettes, vectors, transformed cells and antibodies. Methods include those for expression and detection of BBSR nucleotides and polypeptides.

# FIGURE 1

## Nucleotide and Amino Acid Sequences of Gene X

```

aagccctgaa gggtaaaaag aaatacaaaa gcaaaggcta tttctttttt tttttctttt 60
ctttcattcm ttcccttcctc tgtttctttc tttcttccct tcattttttt ttctttttta 120
agagcgagcg gctctgcggt ggcgggtttgg ggtgggcgcc gccgaggtga ggtcgtctcg 180
cctcccgccg gccggtagat tggttgtttc att atg gat gga ggg gat gat ggt 234
                               Met Asp Gly Gly Asp Asp Gly
                               1                               5

aac ctt att atc aaa aag agg ttt gtg tct gag gca gaa cta gat gaa 282
Asn Leu Ile Ile Lys Lys Arg Phe Val Ser Glu Ala Glu Leu Asp Glu
      10                15                20

cgg cgc aaa agg agg caa gaa gaa tgg gag aaa gtt cga aaa cct gaa 330
Arg Arg Lys Arg Arg Gln Glu Glu Trp Glu Lys Val Arg Lys Pro Glu
      25                30                35

gat cca gaa gaa tgt cca gag gag gtt tat gac cct cga tct cta tat 378
Asp Pro Glu Glu Cys Pro Glu Glu Val Tyr Asp Pro Arg Ser Leu Tyr
      40                45                50                55

```



FIGURE 1 (CONT'D)

gaa agg cta cag gaa cag aag gac agg aag cag cag gag tac gag gaa 426  
 Glu Arg Leu Gln Glu Gln Lys Asp Arg Lys Gln Gln Glu Tyr Glu Glu  
 60 65 70

cag ttc aaa ttc aaa aac atg gta aga ggc tta gat gaa gat gag acc 474  
 Gln Phe Lys Phe Lys Asn Met Val Arg Gly Leu Asp Glu Asp Glu Thr  
 75 80 85

aac ttc ctt gat gag gtt tct cga cag cag gaa cta ata gaa aag caa 522  
 Asn Phe Leu Asp Glu Val Ser Arg Gln Gln Glu Leu Ile Glu Lys Gln  
 90 95 100

cga aga gaa gaa gaa ctg aaa gaa ctg aag gaa tac aga aat aac ctc 570  
 Arg Arg Glu Glu Glu Leu Lys Glu Leu Lys Glu Tyr Arg Asn Asn Leu  
 105 110 115

aag aag gtt gga att tct caa gag aac aag aag gaa gtg gaa aag aaa 618  
 Lys Lys Val Gly Ile Ser Gln Glu Asn Lys Lys Glu Val Glu Lys Lys  
 120 125 130 135

ctg act gtg aag cct ata gaa acc aag aac aag ttc tcc cag gcg aag 666  
 Leu Thr Val Lys Pro Ile Glu Thr Lys Asn Lys Phe Ser Gln Ala Lys  
 140 145 150

ctg ttg gca gga gct gtg aag cat aag agc tca gag agt ggc aac agt 714  
 Leu Leu Ala Gly Ala Val Lys His Lys Ser Ser Glu Ser Gly Asn Ser  
 155 160 165

gtg aaa aga ctg aaa ccg gac cct gag cca gat gac aag aat caa gag 762  
 Val Lys Arg Leu Lys Pro Asp Pro Glu Pro Asp Asp Lys Asn Gln Glu  
 170 175 180

ccc tca tcc tgc aag tct ctc gga aac acc tcc ctg agt ggc ccc tcc 810  
 Pro Ser Ser Cys Lys Ser Leu Gly Asn Thr Ser Leu Ser Gly Pro Ser  
 185 190 195

atc cac tgc ccc tct gct gca gta tgt atc ggc atc ctc cca ggc ctg 858  
 Ile His Cys Pro Ser Ala Ala Val Cys Ile Gly Ile Leu Pro Gly Leu  
 200 205 210 215

ggt gcc tac tct ggg agc agc gac tcc gag tcc agc tca gac agc gaa 906  
 Gly Ala Tyr Ser Gly Ser Ser Asp Ser Glu Ser Ser Ser Asp Ser Glu  
 220 225 230

ggc acc atc aat gcc acc gga aag att gtc tcc tcc atc ttc cga acc 954  
 Gly Thr Ile Asn Ala Thr Gly Lys Ile Val Ser Ser Ile Phe Arg Thr  
 235 240 245

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
2	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74	76	78	80	82	84	86	88	90	92	94	96	98	100	102	104	106	108	110	112	114	116	118	120	122	124	126	128	130	132	134	136	138	140	142	144	146	148	150	152	154	156	158	160	162	164	166	168	170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200
3	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120	123	126	129	132	135	138	141	144	147	150	153	156	159	162	165	168	171	174	177	180	183	186	189	192	195	198	201	204	207	210	213	216	219	222	225	228	231	234	237	240	243	246	249	252	255	258	261	264	267	270	273	276	279	282	285	288	291	294	297	300
4	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92	96	100	104	108	112	116	120	124	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	236	240	244	248	252	256	260	264	268	272	276	280	284	288	292	296	300																									
5	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	215	220	225	230	235	240	245	250	255	260	265	270	275	280	285	290	295	300																																								
6	6	12	18	24	30	36	42	48	54	60	66																																																																																									

aac acc ttc ctc gag gcc ccc tagttttctcc gtccctacac agggagctcc 1005  
Asn Thr Phe Leu Glu Ala Pro  
250

tccccaaggg tagatcggac cgttcattgct gcctataggc attatgtccc tcaaaaaaaaa 1065  
actcctttgc ctgcattcctg tgtacaacat gacattttta accaatccaa tctaaaaaatg 1125  
tgccagaatc cacctgtggc ccgaatcgtg tttgggttcct ctttctactc cactgcagat 1185  
gaccaaaccct gtcccgtgc cactttcctc actgatattg ggaggagggc aaggcccagc 1245  
cgaagtcca ctaaaaatgc cccaggagaa taggcaccgg ctggcttgcc aaagggtttg 1305  
ggttttattg ctttctgttt tttcttttcc cgacagcaca aagaagtaag ggcagttatt 1365  
ggacaggtgt tatttaaaca ttctattgta aatgaatgtg ttgtttggtt ctactgcatt 1425  
gtggagcatg cgggggaaga gaactgacct aggtaatgaa atggagccct tccctggaac 1485  
taaccagtcc ttgatgttgt gtgactaagt aaagatgata aaccccatct gctgggggtg 1545  
tcacttcaca ctggcatgc attgtgaaag ctttccatac ccttggccat tccctctctc 1605  
ctctctctcc aaccccatct atgcaggaag ggactgctaa caagaacgct tccatctcaa 1665  
acctttctc tgccctgggaa attattttat gtttgttttt gaaataaagg atttagttta 1725  
agattctaaa ttttagagaa acaaacgtag gccttgttta ctaatagcca gacatcagaa 1785  
ctgcaggtag gtatgttaat gagatgacct atttctggca gctcctggaa tcctaattatt 1845  
gtaaatgagt gggacacact tgcatattgt gaccattcta ttgaggccct ctctgtttaa 1905  
tgcatattat acttgtgctt ttaactgtgg aatctatttc taacctaaag gtgctgccct 1965  
agtacttttc ttgtctgct ctgctgctct ttttctttc caaacagcaa ctctgaggcc 2025  
atgagcagcc aaaaactaga ggtactgctc cactctgctc cataaaggga aacgggctca 2085  
tcccttggtat tctggaggag ggagaggag atggtgtgga ggctcagag acagagatag 2145  
acatgagctt tgacaacaat ctgtaggctc tccctgctta gaataagcat gtaccattct 2205  
ttatccattc ccttattcc tacatcaatt gtttttactt tcttgggtgt gagactgagt 2265  
gagacacaca caaatgtgt tgacactgt atgccggcag gcagagcagc tactgacttt 2325

## 44

[illegible]

FIGURE 2

Nucleotide and Amino Acid Sequences of  
Plasmolipin Like Protein (PLP)

cgcccgagc	ctgggggaaa	agcggcgcg	gagccggcac	ccaccgctgg	aggggcggcg	60
acggcgccg	tagcgacctc	gggaggcaag	cggagccgcc	atg gcc gag ttc ccg		115
				Met Ala Glu Phe Pro		
				1	5	
tcg aaa gtt agc acg cgg acc agc agt cct gcg cag ggc gcc gaa gcc						163
Ser Lys Val Ser Thr Arg Thr Ser Ser Pro Ala Gln Gly Ala Glu Ala						
	10		15		20	
tcg gtg tcg gcg ctg cgc ccg gac ctg ggc ttc gtg cgc tcc cgc ctc						211
Ser Val Ser Ala Leu Arg Pro Asp Leu Gly Phe Val Arg Ser Arg Leu						

FIGURE 2 (CONT'D)

25	30	35	
ggg gcg ctc atg ctg ctg cag ctg gtg ctg ggg ctg ctg gtg tgg gcg	259		
Gly Ala Leu Met Leu Leu Gln Leu Val Leu Gly Leu Leu Val Trp Ala			
40	45	50	
ctg att gcg gac acc ccg tac cac ctg tat ccg gcc tat ggc tgg gtg	307		
Leu Ile Ala Asp Thr Pro Tyr His Leu Tyr Pro Ala Tyr Gly Trp Val			
55	60	65	
atg ttc gtc gct gtc ttc ctc tgg ctg gtg aca atc gtc ctc ttc aac	355		
Met Phe Val Ala Val Phe Leu Trp Leu Val Thr Ile Val Leu Phe Asn			
70	75	80	85
ctc tac ctg ttt cag ctg cac atg aag ttg tac atg gtt ccc tgg cca	403		
Leu Tyr Leu Phe Gln Leu His Met Lys Leu Tyr Met Val Pro Trp Pro			
90	95	100	
ctg gtg tta atg atc ttt aac atc agc gcc acc gtt ctc tac atc acc	451		
Leu Val Leu Met Ile Phe Asn Ile Ser Ala Thr Val Leu Tyr Ile Thr			
105	110	115	
gcc ttc atc gcc tgc tct gcg gca gtt gac ctg aca tcc ctg agg ggc	499		
Ala Phe Ile Ala Cys Ser Ala Ala Val Asp Leu Thr Ser Leu Arg Gly			
120	125	130	
acc cgg cct tat aac cag cgc gcg gct gcc tcg ttc ttt gcg tgt ttg	547		
Thr Arg Pro Tyr Asn Gln Arg Ala Ala Ala Ser Phe Phe Ala Cys Leu			
135	140	145	
gtg atg atc gcc tat gga gtg agt gcc ttc ttc agc tac cag gcc tgg	595		
Val Met Ile Ala Tyr Gly Val Ser Ala Phe Phe Ser Tyr Gln Ala Trp			
150	155	160	165
cga gga gta ggc agc aat gcg gcc acc agt cag atg gct ggc ggc tat	643		
Arg Gly Val Gly Ser Asn Ala Ala Thr Ser Gln Met Ala Gly Gly Tyr			
170	175	180	
gcc taa acc acc tgt gcc acg gcc ccc tct ggg gct gaa gcc gcc gct	691		
Ala Thr Thr Cys Ala Thr Ala Pro Ser Gly Ala Glu Ala Ala Ala			
185	190	195	
ggg tca cag agc agg gtc acc ctg caa gcc tga agc tgg gga gcc ctg	739		
Gly Ser Gln Ser Arg Val Thr Leu Gln Ala Ser Trp Gly Ala Leu			
200	205	210	
cgt gga gtc agc cca acagggactg catttgctcc tctctgcccg tcagacataa	794		
Arg Gly Val Ser Pro			

1516

# FIGURE 3

## Nucleotide and Amino Acid Sequences of ORPH-PPAR (PPAR)

gtgctgtgag gggcttcggg accttggggc agctcctgag ttcagacaga gttcaggaag 60  
ggagacaggg gcacagagag acagaggttc atggactgag gcaaaggctg ggccaggctc 120  
agcaaccacag gcctcccgca ggcaggcaga ggctgccctg taaccc atg gag acc 175  
Met Glu Thr  
1  
aga cca aca gct ctg atg agc tcc aca gtg gct gca gct gcg cct gca 223  
Arg Pro Thr Ala Leu Met Ser Ser Thr Val Ala Ala Ala Pro Ala  
5 10 15  
gct ggg gct gcc tcc agg aag gag tct cca ggc aga tgg ggc ctg ggg 271  
Ala Gly Ala Ala Ser Arg Lys Glu Ser Pro Gly Arg Trp Gly Leu Gly  
20 25 30 35  
gag gat ccc aca ggc gtg agc ccc tcg ctc cag tgc cgc gtg tgc gga 319  
Glu Asp Pro Thr Gly Val Ser Pro Ser Leu Gln Cys Arg Val Cys Gly  
40 45 50  
gac agc agc agc ggg aag cac tat ggc atc tat gcc tgc aac ggc tgc 367  
Asp Ser Ser Ser Gly Lys His Tyr Gly Ile Tyr Ala Cys Asn Gly Cys  
55 60 65  
agc ggc ttg ttc aag agg agc gta cgg cgg agg ctc atc tac agg tgc 415  
Ser Gly Phe Phe Lys Arg Ser Val Arg Arg Arg Leu Ile Tyr Arg Cys  
70 75 80  
cag gtg ggg gca ggg atg tgc ccc gtg gac aag gcc cac cgc aac cag 463  
Gln Val Gly Ala Gly Met Cys Pro Val Asp Lys Ala His Arg Asn Gln  
85 90 95  
tgc cag gcc tgc cgg ctg aag aag tgc ctg cag gcg ggg atg aac cag 511  
Cys Gln Ala Cys Arg Leu Lys Lys Cys Leu Gln Ala Gly Met Asn Gln  
100 105 110 115  
gac gcc gtg cag aac gag cgc cag ccg cga agc aca gcc cag gtc cac 559  
Asp Ala Val Gln Asn Glu Arg Gln Pro Arg Ser Thr Ala Gln Val His  
120 125 130

ctg gac agc atg gag tcc aac act gag tcc cgg ccg gag tcc ctg gtg 607  
 Leu Asp Ser Met Glu Ser Asn Thr Glu Ser Arg Pro Glu Ser Leu Val  
 135 140 145

gct ccc ccg gcc ccg gca ggg cgc agc cca cgg ggc ccc aca ccc atg 655  
 Ala Pro Pro Ala Pro Ala Gly Arg Ser Pro Arg Gly Pro Thr Pro Met  
 150 155 160

tct gca gcc aga gcc ctg ggc cac cac ttc atg gcc agc ctt ata aca 703  
 Ser Ala Ala Arg Ala Leu Gly His His Phe Met Ala Ser Leu Ile Thr  
 165 170 175

gct gaa acc tgt gct aag ctg gag cca gag gat gct gat gag aat att 751  
 Ala Glu Thr Cys Ala Lys Leu Glu Pro Glu Asp Ala Asp Glu Asn Ile  
 180 185 190 195

gat gtc acc agc aat gac cct gag ttc ccc tcc tct cca tac tcc tct 799  
 Asp Val Thr Ser Asn Asp Pro Glu Phe Pro Ser Ser Pro Tyr Ser Ser  
 200 205 210

tcc tcc ccc tgc ggc ctg gac agc atc cat gag acc tcg gct cgc cta 847  
 Ser Ser Pro Cys Gly Leu Asp Ser Ile His Glu Thr Ser Ala Arg Leu  
 215 220 225

ctc ttc atg gcc gtc aag tgg gcc aag aac ctg cct gtg ttc tcc agc 895  
 Leu Phe Met Ala Val Lys Trp Ala Lys Asn Leu Pro Val Phe Ser Ser  
 230 235 240

ctg ccc ttc cgg gat cag gta cct acc ggc ctg cct gct ggg gag cta 943  
 Leu Pro Phe Arg Asp Gln Val Pro Thr Gly Leu Pro Ala Gly Glu Leu  
 245 250 255

ggc tgg gct ggg gtc agg cgg ccc act cga gtc aac cag aca ggg cac 991  
 Gly Trp Ala Gly Val Arg Arg Pro Thr Arg Val Asn Gln Thr Gly His  
 260 265 270 275

aca cat ccc cac gcc agt atg aat gca cac agc ttg gat ggt gat ggc 1039  
 Thr His Pro His Ala Ser Met Asn Ala His Ser Leu Asp Gly Asp Gly  
 280 285 290

tgg gga cac aca tac ctc tgattcagcg atggctgggg tgcattctcag 1087  
 Trp Gly His Thr Tyr Leu  
 295

ggatggtgac ggtgggggtg catgcatctc tggcacaggg atgatggctcg gggtcacac 1147

ctaggagatg atgatggcta gggacctaca gggcccaggg tcttcttaag ttctggaaga 1207



FIGURE 3 (CONT'D)

ccctcaggcc ctgcagacat tctgtgggta acaagtgacc tgcacaccct gaacaggctg 1267  
 agtggctgac tctaggcccc ctgggagcac aagtgcctac gacttcaggg cttgcatttt 1327  
 agttcaatct ctccagctct gggccatccc tctcggttc taatgggcaa gcagatcttt 1387  
 caggaaaacc aggaggagag gcatgaggag ggtttgaggc cctcagccag tctgtgtgct 1447  
 ggggtggagc aactcagaag agtcaggcca caccacttga atacactcaa cttaggacac 1507  
 tcatgaggca tgtctctgag gctgcccaac ttccaatggc tctgggcgtt cctaaatgtc 1567  
 ccagctgcag ctctggatgg aaccagtggt ctcagatgat aggcagctga gccggatggg 1627  
 gccaaatccc agagctctga gcctctggct gatgtcagga gagcattctc ggggtcccagg 1687  
 acagcacttc cattccttgg gtgcctgaga tgggtggcaga ggctccagac tgagccagag 1747  
 aagctgtgtg tctgccataa caggcacccc tgtctgagca cagggtgatcc tgctggaaga 1807  
 ggcgtggagt gaactctttc tcctcggggc catccagtgg tctctgcctc tggacagctg 1867  
 tcctctgctg gcaccgcccg aggcctctgc tgcgggtggg gcccagggcc ggctcacgct 1927  
 ggccagcatg gagacgcgtg tcctgcagga aactatctct cggttcggg cattggcggg 1987  
 ggaccccacg gagtttgctt gcatgaaggc cttggctctc ttcaagccag agacgcgggg 2047  
 cctgaaggat cctgagcacg tagaggcctt gcaggaccag tccaagtga tgctgagcca 2107  
 gcacagcatt ctagaattaa ggggccgctg aattctaggt taagatggcc cttgacattg 2167  
 agcaggtctt . 2177

NT2 Like Protein (NTPL) Gene  
Fragment Sequence

ACTGNCAGGAACCTCCTGCCACCGCCACCGGCTCCCATGGCCCAATACCCAGTGGGGGTGCCCCAGCAGCGGGGGCAGCC  
CCCATGGGCCCCAGTATTGCGTGTGCAAGGTGGAGCTGTCACTGAGTGGCCAGAACCCTACTGGACCGGGATGTTACCTC  
CAAGTCCGACCCCTTCTGTCTCTTTACAGAGAACATGGCAGATGGATCGAG

Figure 5 - seven-transmembrane protein

DNA

GAATTCCCACAAAGGAGTCCAGGGTCTCGCTCTGTCACACAGGCTGGAGTGC  
AGTGGTGTGATCTTGGCTCATCGT.AACCTCCACCTCCCGGGTTCAAGTGATTC  
TCATGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGTGGTGACTTCCAAGAG  
TGA TCCGTCGGAGGAAAATGACTCCCCAGTCGCTGCTGCAGACGACACTGT  
TCCTGCTGAGTCTGCTCTTCTGGTCCAAGCCAGCGGAACCAGACACACAGG  
AGCAGCCTCCACTACAAACCCACACCAGACCTGCCGATCTCCATCGAGAACT  
CCGAAGAGGCCCTCACAGTCCATGCCCCTTTCCCTGCAGCCCACCCTGCTTCC  
CGATCCTTCCCTGACCCCAGGGGCTCTACCACTTCTGCCTCTACTGGAACCG  
ACATGCTGGGAGATTACATCTTCTCTATGGCAAGCGTGACTTCTTGCTGAGTG  
ACAAAGCCTCT.AGCCTCCTCTGCTTCCAGCACCAGGAGGAGAGCCTGGCTCA  
GGGCCCCCGCTGTTAGCCACTTCTGTACCTCCTGGTGGAGCCCTCAGAACAA  
TCAGCCTGCCCAGTGCCGCC.AGCTTCACCTTCTCCTTCCACAGTCTCCCCAC  
ACGGCCGCTCAC.AATGCCTCGGTGGACATGTGCGAGCTCAAAGGGACCTCC  
AGCTGCTCAGCCAGTTCCTGA.AGCATCCCCAGAAGGCCTCAAGGAGGCCCTC  
GGCTGCCCCCGCCAGCCAGCAGTTGCAGAGCCTGGAGTCGAA.ACTGACCTCT  
GTGAGATTCA TGGGGGAC.ATGGTGTCTTCGAGGAGGACCGGATCAACGCCA  
CGGTGTGGAAGCTCCAGCCC.ACAGCCGGCCTCCAGGACCTGCACATCCACTC  
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CGAACACTCTTCCAGAGGACGAAAGGCCGGAGGGGGGAGGCTGAGAAAGAGA  
CTCCTCCTGGTGGACTTC.AGC.AGCC.AAGCCCTGTTCCAGGACAAGAATTCCA  
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AGCCAACCTCACGGAGCCCGTGGTGCTCACCTTCCAGCACCAGCTACAGCCG  
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GCCCCGGGGCATTGG.AGCAGTGCTGGGTGTGAGACCGTCAGGAGAGAAACCC  
AAACATCCTGCTTCTGCA.ACC.ACTTGACCTACTTTGCAGTGCTGATGGTCTCC  
TCGGTGG.AGGTGGACGCCGTGC.AAAGCACTACCTGAGCCTCCTCTCCTACG  
TGGGCTGTGTGCTCTCTGCCCTGGCCTGCCTTGTACCATTTGCCGCCTACCTCT  
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GCTGGCCGTCTTCTGCTGGACACGAGCTTCCTGCTCAGCGAGCCGGTGGCCC  
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CGTGGTGGAGGTCTTTGGC.ACCTATGTCCCTGGCTACCTACTCAAGCTGAGCG  
CCATGGGCTGGGGCTTCCCC.ATCTTTCTGGTGACGCTGGTGGCCCTGGTGGAT  
GTGGACA.ACTATGGCCCCATC.ATCTTGGCTGTGCATAGGACTCCAGAGGGCG  
TCATCTACCCTTCCATGTGCTGGATCCGGGACTCCCTGGTCAGCTACATCACC  
AACCTGGGCCTCTTCAGCCTGGTGTCTTCTGTTCAACATGGCCATGCTAGCCAC  
CATGGTGGTGC.AGATCCTGCGGCTGCGCCCCACACCCAAAAGTGGTCACAT  
GTGCTGAC.ACTGCTGGGCCTC.AGCCTGGTCTTGGCCTGCCCTGGGCCTTGAT  
CTTCTTCTCCTTTGCTTCTGGC.ACCTTCCAGCTTGTGCTCCTCTACCTTTTTCAG  
CATCATC.ACCTCCTTCCAAGGCTTCTCATCTTCATCTGGT.ACTGGTCCATGC

GGCTGCAGGCCCGGGGTGGCCCCCTCCCCTCTGAAGAGCAACTCAGACAGCGC  
 CAGGCTCCCCATCAGCTCGGGCAGCACCTCGTCCAGCCGCATCTAGGCCTCC  
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 TGTGGCCCCCGAGCCAGGCCAGCCCCAGGCCAGTCAGCCGCAGACTTTGGA  
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 ACCCTGAGGGCACTCTGCATCCTCTGTCAATTTAACCTCAGGTGGCACCCAGG  
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 GGCCCTTTGCCAGGAGCACAGCAGCAGCTCGCCTACCTCTGAGCCCAGGCCC  
 CCTCCCTCCCTCAGCCCCCAGTCCTCCCTCCATCTTCCCTGGGGTTCTCCTCC  
 TCTCCCAGGGCCTCCTTGCTCCTTCGTTACAGCTGGGGGTCCCCGATTCCAA  
 TGCTGTTTTTTGGGGAGTGGTTTCCAGGAGCTGCCTGGTGTCTGCTGTAAATG  
 TTTGTCTACTGCACAAGCCTCGGCCTGCCCCTGAGCCAGGCTCGGTACCGATG  
 CGTGGGCTGGGCTAGGTCCCTCTGTCCATCTGGGCCTTTGTATGAGCTGCATT  
 GCCCTTGCTCACCCTGACCAAGCACACGCCTCAGAGGGGCCCTCAGCCTCTC  
 CTGAAGCCCTCTTGTGGCAAGAACTGTGGACCATGCCAGTCCCGTCTGGTTTC  
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 GAGCCTGACACTCTCCTAAGAGGTTCTCTCCAAGCCCCCAAATAGCTCCAGG  
 CGCCCTCGGCCGCCCATCATGGTTAATTCTGTCCAACAAACACACAGGGTA  
 GATTGCTGGCCTGTTGTAGGTGGTAGGGACACAGATGACCGACCTGGTCACT  
 CCTCCTGCCAACATTCACTCTGGTATGTGAGGCGTGCGTGAAGCAAGAATC  
 CTGGAGCTACAGGGACAGGGAGCCATCATTCTRCCTGGGAATCCTGGAAGA  
 CTTCTGAGGAGTCAGCGTTCAATCTTGACCTTGAAGATGGGAAGGATGTTT  
 TTTTACGTACCAATTCTTTTGTCTTTTGATATTAAGAAAGTACATGTTTAT  
 TGTAAGAAATTTGGAACTGTAGAAGAGAATCAAGAAAGAAAAATAAAAATC  
 AGCTGTTGTAATCGCCTARCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
 AAATAAAAAAAAAAAAAA

## protein

MCELKRDQLLSQFLKHPQKASRRPSAAPASQQLQSLESKLTSVRFMGDMVSFE  
 EDRLNATVWKLQPTAGLQDLHIHSRQEEEQSEIMEYSVLLPRTLFRQTKGRRGEA  
 EKRLLLVDFSSQALFQDKNSSHVLGEKVLGIVVQNTKVANLTPVVLTFFQHQQLQ  
 PKNVTLQCVFWVEDPTLSSPGHWSSAGCETVRRETQTSCFCNHLTYFAVLMVSS  
 VEVDVHKKHYLSLLSYVGCVVSAALCLVTIAAYLCSRRKPRDYTIKVHMLLLA  
 VFLLDTSFLLSEPVALTGSEAGCRASAIFLHFSLLTCLSWMGLEGYNLYRLVVEV  
 FGTYVPGYLLKLSAMGWGFPIFLVTLVALVDVDNYGPIILAVHRTPEGVIYPSMC  
 WIRDSLVSYTNLGLFSLVFLFNMAMLATMVVQILRLRPHTQKWSHVLTLGLSL  
 VLGLPWALIFFSFASGTFQLVVLVLFSTSFQGFLLFIWYWSMRLQARGGPSPLKS  
 NSDSARLPISSGSTSSRI

# DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**HUMAN CHROMOSOME 15 AND 16 BARDET-BIEDL SYNDROME  
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS OF USE**

the specification of which (check one)   X   is attached hereto        was filed on        and was amended on        as Application Serial No.        (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
Number	Country	Day/Month/Year Filed	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 and/or 119(e) of any United States application(s) and/or provisional application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

<u>Application</u> <u>Serial No.</u>	<u>Filing Date</u>	<u>Status</u> <u>Patented, Pending, Abandoned</u>
60/141,753	June 30, 1999	Pending

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code and that such willful false

statements may jeopardize the validity of the application or any patent issued thereon.

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